THE BACTERIAL ORIGIN OF THE GUMS OF THE ARABIN GROUP.

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I.—The Soluble (Arabin) Wattle Gums.

(*Bacterium acaciæ*, n.sp.)

While working upon the gums and slimes produced by some bacteria, it seemed to be exceedingly probable that a few of the gums which occur naturally or are supposed to be formed during a pathological condition of the plant, might have a bacterial origin. Like the mucilages, gums and slimes formed in or on the higher plants, the bacterial slimes are of a varied nature. For example, dextran may be considered as a dextrose anhydride, levan as a levulose anhydride, the gums of Schardinger and Adametz as galactose anhydrides; the bacterium of Marshall Ward and Reynolds Green produces a hemi-cellulose, and Brown's *Bact. xylinum* has a cellulose envelope. The bacterium which I separated from the sugar-cane forms a pentosan slime.

Perhaps the most valuable of the vegetable gums are those of the arabinan-galactan class such as gum arabic and wattle gum; and when one studies the distribution of this kind, it appears to be quite within the bounds of possibility that bacteria have more to do with its formation than would at first appear. Indeed it is extremely probable. In the first place, the gum exudes from cracks* or from punctures or wounds made by insects. The

* "Wattle gum exudes chiefly during the summer season from fissures and accidental injuries to the bark. After careful observation I have formed the opinion that, as a very general rule, it is a pathological product. I came to this conclusion long before I was aware of Trécul's observations that Acacias and the Rosaceæ yield their gums most abundantly when sickly and in an abnormal state caused by a fulness of sap in the young tissues."—Maiden, Pharm. Jour. [3] xx. (1890), 869.

infection of the plant from without is thus indicated. In the second place, every tree does not produce gum. This is, I think, the strongest argument in favour of the mycological origin of the substance. If gum acacia were a natural or even pathological product of the plant itself, one would expect to find it more uniformly distributed than it is. It is not always even uniformly distributed over the tree; some branches may be exuding gum while others are not. The localised positions of gum-bearing Acaciæ are in accord with the theory that gum results from the action of agents, such as bacteria, introduced by insects into the tissues of the plant or by wind-borne dust, laden with bacteria, lodging in a crack or wound.

A recently gathered specimen of wattle gum, which I obtained from Mr. J. H. Maiden, Government Botanist, showed, when examined microscopically, a number of granules which might have been the plasmolysed remains of bacteria. These were not evident in older gums.

Of interest also in this connection is the circumstance that wattle gum has nutritive properties,* and that O'Sullivan found a proteid in samples of Gedda gum. It is not at all improbable that this proteid substance was the remains of bacterial cells.

It would be useless to attempt the isolation and cultivation of bacteria from fragments of dry gum, because any micro-organisms that had been there would, during the process of drying, have been killed plasmolytically. Fresh material must be investigated, and to obtain this I applied to Mr. Maiden, who referred me to Prof. Liversidge, in whose paddock at Mittagong he had seen a specimen of *Acacia penninervis* bearing a quantity of gum. Prof. Liversidge sent me a gum-bearing branch, and this was investigated.

From the twigs small portions containing a drop of gum were cut; from these the gum was removed and the part sterilised by rapidly passing it through a bunsen flame. Smaller parts of these

* Maiden, Proc. Roy. Soc. N.S. Wales, xxxv., 171.

portions were inserted into faintly acid saccharose-peptone media* and incubated at 30°. From the tubes of media that became turbid, tubes of saccharose-peptone agar were infected and plates prepared in the usual manner. The media infected with fragments of heart-wood were sterile, but in cases where the bark had been taken, two kinds of colonies developed. One of these was in the minority, and will be referred to again in dealing with the insoluble gums. The prevailing bacterium was stroked upon saccharose agar, and the growth was found to contain capsulated bacteria together with slimy bacterial clumps. A bacterium had therefore been isolated which promised well for the research.

A number of experimental cultures were then made with the object of determining the constituents which might be employed to construct a prescription for the manufacture of a medium that would yield a maximum amount of slime and at the same time enable one to trace the constituents from which the slime is derived. The experiments, however, led to no useful result, partly because too high a temperature (30°) was employed and partly because a discrimination was not made between the quantity of bacteria and the amount of slime. The question of the slime-forming constituents will be discussed in a future paper. On a peptone-saccharose agar medium, however, enough slime was obtained to enable a few tests to be made.

The slime, which adhered to the surface of the medium, was moistened with water, and after it had swelled it was removed with a rubber spade, the greatest care being taken to avoid removing portions of the agar.

The slimy emulsion was stirred with a few drops of dilute hydrochloric acid and precipitated with alcohol. The coagulum, on treatment with water and then alcohol, produced an opalescent solution from which the slime was precipitated by potassium chloride. The watery emulsion was found to be free from

* An improvement upon this method of isolation would be to employ ordinary glucose gelatine, in which the bacteria grow very well. Tubes of molten glucose gelatine should be infected with the fragments of wood and plates formed from these at once and after an incubation of 1, 2, 4, 8 and 24 hours at 30°.

saccharose and reducing sugars, and was used for the test-tube reactions. Flocculent white precipitates were obtained with basic and ammoniacal lead acetates. Neutral lead acetate gave no reaction. Neither did the salts of silver and mercury. The hydrates of calcium and barium gave no precipitate. Copper sulphate followed by potassium hydrate gave a flocculent precipitate which became cohesive on boiling and did not darken in colour. When tested at a later date by the method recommended by Maben,* borax stiffened the mucilage, while basic lead acetate and also ferric chloride hardened the gum acids forming cohesive masses. Tannic acid had no effect beyond producing an opalescence.

The natural gum of *Acacia penninervis* is not entirely soluble in water; the major portion simply swells up to form gelatinous lumps.† The portion that does dissolve acts towards the salts of

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* Maben, Pharm. Jour. [3] xx., 719.

† "*Acacia penninervis*, Sieb., 'B. Fl.' ii., 362.

A "blackwood" and "mountain hickory." Found in Tasmania, Victoria, New South Wales and Queensland. Sample from Quiedong, near Bombala, New South Wales.

Arabin	70·3
Metarabin	11·42
Moisture	16·67
Ash	0·66

While not approaching the best kinds of gum arabic in lightness of colour, it is the palest and cleanest looking of the coast Wattle gums described in this paper. Colour pale sherry to orange. It has a bright fracture, although a sample received from Brown's Camp, Delegate, and obtained from various trees 6 feet 2 inches, 6 feet in diameter and 40 feet to 60 feet high, has a dull fracture like *A. binervata*. It is interesting to note that in the Quiedong district the gum was obtained in abundance from shrubs or small trees growing in low ground, while in the Braidwood district no gum whatever could be found on trees of this species, where it grows at high elevations, and at Delegate, at moderately great elevations, only very small quantities of gum could be obtained. The inference is that this species yields gum plentifully from shrubs at low elevations, but little or none from trees at high ones. The same thing has been found to hold good to some extent in regard to *A. dealbata*. These observations point to the truth of the oft-expressed surmise that gum is a pathological product or the product of plants in uncongenial surroundings.'—Maiden, Pharm. Jour. [3] xx. (1890), 980-1.

copper, lead, silver and mercury and to the hydrates of calcium and barium in a manner precisely similar to a solution of commercial gum arabic and to the bacterial slime.

The next step was the testing of the slime for pentosans. A portion was distilled with hydrochloric acid of sp.g. 1·06, and the distillate on treatment with a hydrochloric acid solution of phloroglucin yielded the furfural reaction. Another portion was carefully heated with strong sulphuric acid, and the volatile products gave the furfural rose colour to paper moistened with aniline acetate. These reactions were also obtained with portions of the natural gum of *Acacia penninervis*. The bacterial slime, therefore, contained pentosans as evidenced by the production of furfural on treatment with acids.

As I have already said, the cultures upon an artificial medium made up from a few constituents were not very successful. This medium contained saccharose, peptone, glycerine, potassium phosphate and chloride, and agar in proportions that experiment seemed to indicate as being suitable. Agar must be employed, because, although on all gelatine media masses of slime enclosing bacteria can be observed, yet, as the medium is slowly liquefied by the bacterial products, the slime would be contaminated with peptonised gelatine. In the course of experimenting with various media, potato-extract agar gave a good growth, and this was increased by the addition of glucose or saccharose. But even this growth was not what would be called rich in gum or slime.

At this stage several observations were made. After scraping the culture from the surface of saccharose-potato agar, the plates, which had been incubated at 30°, were allowed to stand at the laboratory temperature (15°) for several days, when a quantity of slime greater than the original crop at 30° was obtained. This appeared to indicate that a lower temperature and a slower growth was accompanied by relatively greater slime formation. In separating the bacteria from the tissues of a specimen of gum-

bearing *Acacia binervata*, DC.,* it was noted that subjacent to the hole in the bark through which a large globule of gum had exuded there occurred a small quantity of fluid which was acid and contained a tannin body. In the tissue saturated with this fluid, slime-forming bacteria identical with the bacterium from *Acacia penninervis* were obtained in pure culture. The occurrence of tannin at the site of production of the gum suggested that it might influence the production of gum. Accordingly experiments were made with it, and from these it appeared that from 0·2 to 0·4% of tannin did influence the formation of slime. Smaller proportions of tannin when added to saccharose-potato agar seemed to produce a relatively greater quantity of bacteria and less slime, while more tannin produced less bacteria and less slime. With regard to the reaction of the medium, experiments made with varying amounts of citric acid and of sodium carbonate

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* This was growing in a garden in Macpherson-street, Waverley, Sydney, and my attention was drawn to it by Miss S. Hynes, of the Botanic Gardens.

Maiden (Pharm. Jour. [3] xx., 980) gives the following description of the gum:—

"*Acacia binervata*, DC., B.F. ii., 390.

Arabin	76·57
Metarabin	4·24
Ligneous matter	1·62
Moisture	16·01
Ash	·771
	99·211

"A 'black wattle' found in New South Wales and Queensland; obtained from old trees, Cambewarra, New South Wales; diameter 8-12 inches. It is obtained in pieces from the size of a pigeon's to that of a hen's egg, and is of a waxy lustre. The freshly exuded gum is very pale brown; old gum is often nearly black. Being in comparatively large masses, sorting for market would be easy. It has a dull horny-looking fracture.

It dissolves fairly well in water, leaving a quantity of gum in the form of a flocculent deposit. The colour of the dissolved gum is rather dark owing to the presence of included fragments of bark. This description of the effect of cold water will apply equally well to *A. dealbata* and *A. elata*. Like other wattle gums, its aqueous solution is distinctly acid to litmus paper."

showed that acidity or alkalinity of the medium favoured neither the growth of bacteria nor the production of slime.

As the function of tannin might consist in slowing the growth of the bacteria, glycerine which appeared to do the same thing, was also employed in an experiment to test the formation of slime.

Several plates of saccharose-potato agar which contained 0·06 grm. tannic acid or 1 c.c. glycerine in every 20 c.c. of medium were prepared. The agar surfaces were smeared with a culture of the bacterium, and the plates were incubated at 22° and 30° for three days. After noting the appearances of the cultures, the slimes were removed with a rubber spade, and after mixing with water slightly acidified with hydrochloric acid, alcohol was added. The compact or flocculent precipitates were, after washing with alcohol, dried at 100° and weighed. The results are summarised in the following table :—

Addition to medium.	Incubation temperature.	Nature of growth.	Order as judged by the eye.	Mgrms. crude dry slime.
Glycerine.	22°	Granular, adherent, yellow paste.	2	58
,,	30°	,, ,,	4	61
Tannic acid.	22°	Pale buff slime, non-adherent.	1	83
,, ,,	30°	Buff paste non-adherent.	4	38

The results show that it might be possible to obtain a quantity of slime by using tannic acid and by using the lower temperature. They also show how one can be misled by appearances, for there appeared to be much more growth produced on the glycerine medium at 22° than at 30°, while in reality there was a little less.

On tannin agar the production of slime appears to have finished in from four to five days at 22° C., and the medium assumes a blackish-green colour. If the plate had not been uniformly smeared, and the growth in consequence had formed irregularly, it is noticed that the culture and medium are buff coloured at the margin, and dirty green in the middle where the growth is

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older. The change from buff to green results from the alteration in the reaction of the medium from neutral or faintly alkaline to decidedly alkaline, as is shown by pressing litmus paper against parts of the under side of the plate—*i.e.*, the agar in contact with the bottom of the petri-dish. The greenish, mottled slime is converted to a buff colour by the addition of a few drops of dilute hydrochloric acid.

When the culture becomes dark-coloured there is not so much slime obtained; what has been formed appears to have condensed and to have become less soluble in water, so that the culture forms a suspension with water, while the earlier buff cultures form a stiff slime under similar conditions.

Further experiments with the tannin medium showed that the slime was formed more readily by growing the bacteria at 15°—the laboratory temperature at the time—than at 22°. The most successful method consisted in growing the culture at 15° for three days, and then scraping the slime from the plates. In another two days a further quantity can be removed, and possibly still another in two days later. The slime is acidulated with a few drops of dilute hydrochloric acid and treated with alcohol, when either white stringy flakes or white floccules are precipitated according to the alcohol which has been added. The stringy, cohesive flakes are changed to floccules by strong alcohol. The alcoholic mother liquor is coloured a bright yellow from the lipochromes of the bacterial cells. The floccules of slime and bacteria are white, and on treatment with water swell up, forming a stiff paste like that made from flour. The opalescence is caused by the bacteria, and to eliminate them the paste was treated with 2 to 5 drops of dilute sulphuric acid, and heated in the autoclave at 3 atmospheres' pressure for 15 minutes. This treatment had been found very useful in separating bacteria from slime on a former occasion. By this treatment a faintly opalescent, gummy fluid, which could easily be separated by filtration through paper from the precipitated bacteria, was obtained. The faintly opalescent fluid was easily clarified with aluminium hydrate. The difference between slime and gum appears to be caused by the

presence of albuminoids secreted by or contained in the bacteria. When the albuminoids are coagulated by treatment in the autoclave the slime is altered to a gum.

Having indicated the method for obtaining a solution of the gum acids free from dead organisms, a word may be said about the bacteria. The bacterium from *Acacia penninervis* was identical with that separated from *A. binervata*, but while with the former another bacterium was associated, the bacteria in the latter were in pure culture. The bacteria, however, were of different races—indicated only by the production of larger colonies on nutritive media and more slime on tannin-saccharose-potato agar. The stronger race (from *Acacia binervata*) was used subsequently for the production of the artificial gum.

The medium upon which most slime was obtained consisted of

Potato extract	1,000 c.c.
Saccharose	50 grms.
Agar	20 ,,
Tannic acid	3 ,,

The potatoes were washed, pared, eyed, grated and finally strained and pressed in a meat-press. The juice was then allowed to stand overnight in a flask of such a size that the juice filled the neck. The darkening of the fluid by contact with air was thus minimised. In the morning the juice was siphoned off from the starch and filtered. An equal volume of water was added to the filtrate, which was then boiled to coagulate the albuminoids, which were removed by filtration from the potato-extract. To the extract thus obtained, the sugar was added and the solution heated in the autoclave to three atmospheres' pressure* in order to kill the spores of *Bac. levaniformans*, which are generally present in commercial sugar. After removal from the autoclave, the chopped agar was added and the solution was returned to the autoclave and heated to one and a half atmospheres' pressure to bring the agar into solution The tannin was then added and the medium was steamed for an hour, after which it was cooled to 50° and poured into sterilised (by flaming) large damp-chambers or small

* This would be unnecessary if dextrose were used.

petri-dishes. The medium in the larger vessels was after congelation infected with a suspension of the bacteria in 10 c.c. of normal saline; the medium in the petri-dishes, by smearing with a loop of the culture. The culture used for the purposes of infection was obtained by growing the bacteria at 30° in potato-saccharose agar prepared in the same way, but without tannin. The incubation at 30° on this medium gives a loose yellow growth with very little slime; the culture therefore readily becomes distributed in the normal saline. The plates were kept at the laboratory temperature (about 15°) and the slime was removed with a rubber spade on the third, fifth and seventh days. The slime is easily removed, and there is no danger of agar being taken at the same time. The first and second crops were preserved by the addition of a few drops of dilute hydrochloric acid and of alcohol until all the slime was obtained. The total slime was then treated with an excess of strong spirit and worked up in the manner already described. The difference in the amount of slime obtained by adding tannin to the potato-saccharose agar is very marked. Without tannin the growth is bright yellow and slightly gummy, while with tannin it is pale buff, thick and slimy.

A portion of the gummy solution free from sugars was precipitated with alcohol and the precipitate treated with nitric acid, sp.g. 1·12. A white, sandy powder, difficultly soluble in cold water but readily soluble in boiling water, was obtained. It had an indefinite melting point over 210° C., and had the same crystalline appearance, viz., colourless tables with a straight side, as mucic acid obtained from commercial gum acacia. The mother liquor contained oxalic acid. The oxidation products are thus identical with those of gum acacia.

So far the identity of the bacterial gum with the natural gum has been proved by the test-tube reactions, the presence of pentosans and the oxidation products. There remained the optical activity and the determination of the constituents of the gum acids. The former, viz., the optical activity, is of little consequence in view of the researches of other investigators.

O'Sullivan* has shown that fragments of the same gum contain gum acids which rotate the ray of polarised light to different extents. He also showed that different kinds of gum of the arabin group have rotations in different directions; for example, gum arabic is lævo-rotatory, Gedda gum is generally dextro-rotatory, and an Australian gum was optically inactive. It is therefore probable that the gum acids of the natural gum and those of the bacterial gum would differ in their optical activity, since the conditions under which the gums had been produced had been so very different. This proved to be the case. I prepared the gum acids from the gum of *A. binervata* and from cultures of *Bact. acacice* and submitted them to Mr. T. U. Walton, B.Sc., of the Colonial Sugar Refining Co., who found that the natural gum acids had a specific rotation of $[a]_D = +0°{\cdot}9$ and the bacterial gum acids had a rotation of $[a]_D = +43°$. I do not lay any stress upon the difference in the optical activities. In the future I shall grow the bacterial gum in different ways and from different materials, to see how the optical activity is thereby influenced.

A portion of the sugar-free gum acids was dissolved in 15 c.c. of water, and to this 10 c.c. of 5 % sulphuric acid was added, thus making a 2 % solution. This was heated on the water bath for an hour to hydrolyse the combined arabinan. Subsequent work showed that this heating might with advantage be prolonged for three or four hours to hydrolyse the arabinan more completely. After the digestion with the 2 % acid the solution was cooled and the unaltered gum acids precipitated with alcohol. The solution, after filtration, was distilled until most of the alcohol had been removed; the residual fluid was, after the addition of about 50 c.c. of water, neutralised with barium carbonate and filtered. The filtrate was boiled down to one-half and clarified with aluminium hydrate. The clear solution was used for the preparation of the osazone.

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* O'Sullivan, Journ. Chem. Soc. 1891, 1029.

The residual gum acids which did not hydrolyse by the 2 % acid treatment were dissolved in 25 c.c. of 5 % sulphuric acid and warmed to expel the traces of alcohol. The solution was then made up to 25 c.c. with water and boiled under a reflux condenser, at first gently and then vigorously after the foaming had ceased. The boiling was continued for five hours. After cooling, the acid solution was treated with alcohol and a very small quantity of unaltered gum removed. The alcoholic solution was treated as has been already described, and the final solution reserved for the preparation of the osazone.

The solution supposed to contain arabinose was tested with Fehling's solution, when a strong reduction was obtained. One-half of the solution was set aside in case of accidents. The other half was heated on the water bath and to it were added 2 c.c. of a solution containing 40 c.c. phenylhydrazine, 40 c c. glacial acetic acid and 20 c.c. water. The heating was continued for an hour and the solution was cooled. The precipitated osazone was filtered off, dried on porcelain, transferred to a small filter and extracted with ether* to remove a black, tarry substance which melted at about 98°. This was always found in the osazones from the bacterial as well as the natural gums. It was never obtained in working with pure sugars such as dextrose. The precipitate was dried at 100° and the melting point determined. This was 160°, which, together with the appearance of the crystals, showed it to be the osazone of arabinose.

The solution, which presumably contained galactose, reduced Fehling's solution. One-half was treated with phenylhydrazine-acetic acid and the osazone extracted with ether. The dry osazone melted at 182-183°, which showed it to be a mixture. The mixed osazone was boiled with 10 c.c. of water† and filtered through a hot filter which had been moistened with boiling water.

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* A preliminary moistening with alcohol is sometimes advantageous.

† In some cases the osazones could not be separated by water alone, but by dissolving everything in dilute alcohol and slowly boiling off the alcohol, the galactosazone precipitated out from the hot solution and was separated by filtering through a hot, wetted filter.

The filtrate on cooling deposited microscopic crystals which dried as an Arabian-brown skin on porcelain and which melted at 157-159°, showing them to be the osazone of arabinose. The portion insoluble in water melted at 187°. This was again treated with 10 c.c. of boiling water. The insoluble portion dried as a yellow powder and melted at 192-193°, the melting point of the osazone of galactose.

The bacterial gum acids had therefore yielded arabinose and galactose on hydrolysis, from which we must conclude that they contain the arabinan-galactan complex and are of the same nature as the natural gums of the arabin group.

The gum which was found upon *Acacia binervata* was of a very pale yellowish-brown and of a dark brown colour. Portions of the same mass showed both colours. It occurred in hemispherical masses and in tears and had a tough gelatinous consistency which enabled it to be cut without fracture. It dissolved readily in water, and in the absence of particles of bark it formed a clear solution with an acid reaction. The acidity of 100 grms. of gum to litmus paper was equal to 3·27 c.c. of normal acid.

Although there could be no doubt that the gum contained the arabinan-galactan complex, yet to complete the identity of the bacterial with the natural gum acids the proof was needed. Accordingly a portion of the natural gum was dissolved in water, acidified with hydrochloric acid and treated with alcohol. The gum acids were hydrolysed and the sugars tested by means of their osazones in the manner already indicated, when arabinosazone and galactosazone were obtained. Furthermore, like the bacterial acids, there is apparently a greater proportion of arabinan than galactan in the complex.

Summary.—A bacterium was found in pure culture at the place from which the natural gum was exuding. This bacterium in the laboratory formed a gum which behaved to reagents, gave the same oxidation products, and contained the same constituents, viz., arabinan and galactan as the natural gum. The bacterium is thus the producer of the natural gum.

The bacterial origin of that variety of gum acacia exuding from *Acacia binervata* having been proved, it is a just assumption that all other gums of the arabin group are likewise bacterial products, and not substances formed by the plants in a pathological condition.

The formation of gum is, therefore, a bacterial disease, for the parasitic bacteria obtain their nourishment from the plant juices which they elaborate into gum, which is not required by the host plant. It is still undecided what constituents they may alter. Gum can be formed from saccharose and from dextrose, but I will deal with this side of the subject in a future paper.

The bacteria that are parasitic in plants are usually found in the feebly acid or neutral juices of the vessels, and probably the gum bacteria are located in the sieve tubes of the soft bast, as Kraus * has indicated, although the gum is not a true cell content as he supposed.

Bacterium acaciæ, n.sp.

Shape, etc.—The bacteria appear as short rods with rounded ends, and occur singly, in pairs, and in groups, sometimes within a well-defined slime case (ascus). On nutrient agar the rods measure 0·5 : 1 μ; in bouillon 0·5 : 1·5-2 μ, pairs commonly occur simulating a long rod; on glycerine-saccharose agar the bacteria vary from 0·5 : 0·5-1·5 μ, appearing as cocci and as short rods; on saccharose-potato agar they measure 0·6 : 1-2·5 μ and average 0·6 : 1·5 μ. The cells stain well with the ordinary stains;

* "Herr G. Kraus has determined by observations on the exudation of gum from *Acacia melanoxylon* that it is formed only in the bark and not in the wood, and only in the bast layer, never in the parenchyma nor in any more external portion that the bast fibres have no share in its formation; that it flows from the cells of the soft bast, and especially from the sieve tubes, and that it is not a product of degradation of the cellulose but is a true cell content flowing out unchanged through the unchanged cell walls." Abstract in Journ. Roy. Micr. Soc. [ii.] vi. (1886), 90, from Ber. Sitz. Naturf. Gesell. Halle, 1884, pp. 19-20.

the Gram stain is negative. The bacteria are actively motile; the flagella vary from one, terminal, to many, peritrichous; in the latter cases up to six have been observed, but there may be more.

Temperature, etc.—The optimum temperature is apparently about 22° on saccharose-potato agar; the growth is most bulky at 22°, then at 15°, then 30°, and smallest at 37°. The organism is aërobic, but grows scantily under anaërobic conditions.

Nutrient agar plate.—At 30° the colonies are circular, white, slightly raised, and moist glistening. When magnified they are seen to have a smooth circular edge and contain scattered granules. The deep colonies are irregular, oval or round, and coarsely granular. The colour changes from white to primrose yellow.

Glucose-gelatine plate.—The colonies are white, circular but sometimes lacerate, slightly raised and glistening like drops of gum. The colour deepens to yellow. When magnified, granules are seen scattered throughout the colony, and as growth proceeds these become coarser and more numerous. The deep colonies are at first dark, rough and irregular, but become rounded or moruloid. The old surface colonies are raised, slimy and streaky. The medium is very slowly liquefied.

Nutrient gelatine plate.—As on glucose-gelatine, but the growth is not so good.

Nutrient agar stroke.—A white, flat, moist, glistening stroke is first formed; this changes to a primrose or yellow, dry, terraced growth. The margin may be straight or lobed.

Saccharose-potato agar stroke.—The temperature of incubation has a great influence upon the appearance of the cultures. In 3 days at 30° the growth is yellow-buff, opaque and terraced. At 25° the stroke is transparent at the margin and streaked with white in the centre, while the growth has flowed downwards into the condensed water, which has become a thick slime. At 22° the opacity and growth is more pronounced, the colour is pale buff. The growth is more undulating both at 15° and at 22°

than at 30° or 37°. At 37° the growth is as at 30° but more scanty.

Glucose-gelatine stroke.—The growth is irregular, spreading, slimy, and becomes depressed in places owing to the slow liquefaction of the medium. The colour changes from white to canary-yellow.

Nutrient gelatine stroke.—As on glucose-gelatine but poorer.

Glucose-gelatine stab.—A strong filiform growth with a translucent, white, flat, slimy nail-head. The nail-head slowly sinks, and a funicular, then stratiform, liquefied area is formed. The medium may develop a few gas bubbles. Saccharose is also fermented with an evolution of gas. Some races liquefy the medium slowly, and an air-bubble is produced at the top of the filiform stab. Taken as a whole the liquefaction of the medium is slow.

Nutrient gelatine stab.—A smaller growth than glucose-gelatine.

Potato.—The growth is at first dull buff, raised and irregular, then becomes glistening and deep yellow.

Bouillon.—The medium becomes turbid, and forms a loose flocculent sediment, and a thin broken surface film which adheres to the sides of the glass. The sediment becomes deep yellow and cohesive. The recently isolated races produced indol together with nitrite in from 10 to 15 days at 30°. After the races had been cultivated in the laboratory for several months the red nitroso-indol reaction was in some cases faint, and in other cases was absent. In the bouillon ammonia could be detected, but neither phenol nor sulphuretted hydrogen.

Milk.—The medium is either unaltered or made slightly ropy with a faint acid reaction.

The bacterium has characters which mark it as being new, the most important of these, being the production of arabin. I accordingly name the organism *Bacterium acaciæ* (*Bacillus acacie* by Migula's system).

II.—The Insoluble (Metarabin) Wattle Gums.

(Bacterium metarabinum, n.sp.)

While the natural gum of *Acacia binervata* was, in the absence of particles of bark, entirely soluble in water, the gum of *Acacia penninervis* was only partly so. The bacteria associated with the gum of the former were entirely *Bact. acaciæ*, while from the latter this organism and another were found. As separated from my culture solutions the other bacterium was in the minority, but on considering the matter this can be accounted for by its method of growth. *Bact. acaciæ* in peptone saccharose fluid grows as single cells, while the other bacterium has a tendency to grow in aggregates*, and thus in plate culture the aggregate comés out as if it had been a single cell.

I did not think that the plant would convert soluble gum to the insoluble or meta modification, and expected that the insolubility resulted from the further action of the organism, the action of tannin, or that it was produced by quite another bacterium. The first is unlikely, the second is quite possible, and the last is most probable. Tannin is known to stiffen the gum, and it would therefore be an easy thing to affirm that the insolubility was entirely due to its action. But the gum of *Acacia binervata* is formed in the presence of tannin bodies, and although fragments of bark are imbedded in the solid exudate, yet, when these are picked out, the gum is found to be entirely soluble in water. There is, therefore, strong reason to believe that tannin has nothing to do with the production of the insoluble portion of the gums.

The second bacterium from *Acacia penninervis* forms, in suitable media, slime masses similar to *Bact. acaciæ*, and while the latter forms gummy colonies on gelatine media, the colonies of the former are dry and cohesive, so that the colonies are removed from the medium by the inoculating needle *en masse*. There is

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On this account the bacterium must be "plated" several times before a pure culture is obtained.

in this method of growth a strong indication that the slime is more insoluble than that secreted by *Bact. acaciæ*. On saccharose-potato agar with or without tannin *Bact. acaciæ* produces at 15° a gummy slime that does not adhere firmly to the medium. The other bacterium forms a thicker slime, and the portion in contact with the medium adheres firmly and cannot be entirely removed with a rubber spade. When the two slimes are carefully removed, dried in the steam bath at 100° and then moistened with water, the slime of *Bact. acaciæ* swells up, forming a diffusive slime very similar to its original condition, while the slime of the other bacterium smells slightly and retains the shape in which it had dried. There is thus a strong probability that the new bacterium forms the insoluble gum (the meta-arabin) of wattle gum. That the slime might eventually prove to be of the arabinan-galactan class was indicated by the great similarity of the growth on tannin-saccharose-potato agar with that produced by *Bact. acaciæ*.

The nature of the slime had now to be determined, and as with *Bact. acaciæ*, large plates of tannin-saccharose-potato agar were infected with a suspension of the bacteria in 10 c.c. of normal saline, and these were kept at the laboratory temperature (15°). The slimes wers collected, rendered more acid with a few drops of dilute hydrochloric acid and treated with alcohol. A curdy precipitate which became cohesive and an opalescent solution were obtained. The precipitate was stirred repeatedly with alcohol until no further opalescence was produced. The precipitate was treated with water and then with alcohol, when a thin starch paste-like suspension was produced. This was flocculated with barium chloride (potassium chloride did not act so well as the barium salt), and a contractile curd separated from an opalescent solution. The opalescent alcoholic solution was added to the opalescent solution previously obtained and the gum in this was precipitated by the addition of barium hydrate and reserved for future examination.

The contractile curd was treated with water, with which it formed a thick paste. The lumps were broken up by passage through a wire-gauze filter and the whole was warmed to cause

a uniform swelling. It had an acid reaction to litmus paper. By heating in the autoclave for ten minutes at 3-4 atmospheres a slimy sediment (apparently gummy) and an almost clear, slightly acid, supernatant fluid was produced. The slimy sediment was mixed with water treated with 2-3 drops dilute sulphuric acid and again heated in the autoclave. This gave a curdy precipitate and supernatant solution. The curdy precipitate was apparently free from gum, and as it probably consisted of coagulated bacteria it was not examined further. The faintly acid solution from the first autoclave treatment was tested with a few reagents. Lead acetate, barium hydrate and barium chloride gave no precipitate. Basic lead acetate and ammoniacal lead acetate gave precipitates. Fehling's solution gave no precipitate and no reduction on heating. Copper sulphate followed by sodium hydrate gave a precipitate which coagulated on boiling without change of colour. Alcohol gave an opalescent solution which flocculated readily with potassium chloride.

The gum acids in the mixed solutions from the autoclave treatment were precipitated with alcohol and potassium chloride and kept over-night in contact with the alcohol. The precipitate was very contractile, and on treatment with water it partly dissolved and partly swelled up, forming a practically unfiltrable suspension. A portion of this was preserved; it had not dissolved at the time of reading this paper, *i.e.*, in one month; the gelatinous lumps were still evident. We have in this insolubility of the gum acids a condition identical with what occurs on treating the semi-insoluble wattle gums with water, and confirms the deduction made from other observations that this bacterium is responsible for the production of the metarabin of these gums. The gelatinous lumps of gum acids were insoluble in dilute acid, but readily dissolved in dilute sodium hydrate, from which dilute hydrochloric acid precipitated the gum acids.

The acids were dissolved in dilute sodium hydrate, neutralised with sulphuric acid, and enough 5 % sulphuric acid was added to make a 2 % solution. This was boiled under a reflux condenser for two and a half hours. A white precipitate which contained

no gum acids and proved to be barium sulphate was filtered from the dilute sulphuric solution. Alcohol did not produce a precipitate in a portion of the filtrate, so that the gum acids apparently had been completely hydrolysed by the treatment. The solution was neutralised by boiling with barium carbonate, filtered and the filtrate clarified with aluminium hydrate. A portion of the solution was treated with phenylhydrazine acetate solution, and a mixed osazone was obtained. When the constituents were separated in the manner indicated in the first portion of this paper, they proved to be arabinosazone (m.p. 158-160°) and galactosazone (m.p. 192-193°). The gum acids therefore contained the arabinan-galactan complex, and the gum belonged to the arabin group.

The opalescent alcoholic solution which coagulated on the addition of barium hydrate was washed by suspension in alcohol and finally treated with water, in which it formed a semi solution. Dilute sulphuric acid was added until no further white precipitate formed, and the suspension was heated in the autoclave, when a sediment of barium sulphate and a supernatant fluid was obtained. From this, alcohol threw down a small quantity of a contractile precipitate which dissolved easily in water. On testing the solution, which was acid to litmus paper, precipitates were obtained with basic lead acetate, ammoniacal lead acetate and barium hydrate. Copper sulphate followed by potassium hydrate gave a precipitate which contracted but did not darken on boiling. Barium chloride, sodium hydrate, lead acetate and Fehling's solution gave no precipitates. The reaction with barium hydrate suggests the possibility that the compound may be a pectin substance.* On hydrolysis with sulphuric acid

* The pectin substances are very indefinite and yield on hydrolysis various sugars. Bourquelot found that gentian pectose hydrolysed to arabinose and doubtful crystals of galactose. Hébert obtained arabinose from the pectic bodies of ripe fruit and bulbs, but as they also yielded mucic acid on oxidation, he considered that galactan had been present. Bauer hydrolysed apple pectin to xylose and pear pectose to galactose. Herzfeld concluded that parapectic acid contained arabinan and galactan.

arabinose and galactose were obtained. These were identified as in the other cases by the isolation of the osazones and the determination of the melting points. The same substance was not obtained from the natural gum of *Acacia penninervis*, but this may be accounted for by the small quantity of the gum which I had at my disposal at that time.

The growths in test-tubes containing saccharose-potato agar with and without tannin apparently show that tannin has little effect in increasing the quantity of slime. The difference, however, is more marked in plate cultures. In one case the slime scraped off from two similar large plates weighed when dry:—tannin 1·624 grm., without tannin 1·180 grm.

In purifying the gum acids it was noted that hydrochloric acid prevents the precipitation with alcohol much more than in the case of the gum acids of *Bact. acaciæ*, and sometimes a strong flocculating agent, *i.e.*, barium chloride, has to be employed. Again, the separation of the bacteria from the gum acids has to be very carefully done if one would obtain the acids in the meta condition. In a second case, when a few drops of dilute sulphuric acid were added and the heating in the autoclave continued for a longer period (45 minutes) the gum acids, after precipitation with alcohol, dissolved readily in water. The insolubility was, however, restored by heating the dried gum acids at 100° for two hours. After this treatment they did not dissolve entirely in water, the floccules being visible after five days. The gum acids of *Bact. acaciæ*, when heated at 100° for the same time, dissolved in water as readily and in the same manner as commercial gum acacia.

The natural gum of *Acacia penninervis*, like the bacterial gum, contained the arabinan-galactan complex.

Summary.—The bacterium which I have named *Bacterium metarabinum* * was found at the place from which the gum was exuding. It produces a gum which is tough and gelatinous, as evidenced by the consistency of the colonies on nutritive media.

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* *Bacillus metarabinus*, by Migula's system of nomenclature.

The gum yields gum acids which partly dissolve* and partly swell up with water. These have the test tube reactions of gum acacia and contain the arabinan-galactan complex. The gum acids are similar to those of the natural gum of *Acacia penninervis*, and there can be no doubt that the natural gum is of bacterial origin. Since *Bact. acaciæ* was found with *Bact. metarabinum* the gum is probably produced by both bacteria, the former producing the water-soluble portion, the latter the water-insoluble portion.

Since *Bact. acaciæ* was found in the only two instances which were examined, it is possible that it is the only producer of all sorts of soluble acacia gums. If this should prove to be the case, the varieties of the gums will depend upon a number of factors such as the host plant, the juices of which undoubtedly vary, and thus influence the relative proportions of arabinan and galactan in the gum complex. The situation will undoubtedly influence the susceptibility of the tree towards the microbe. It is generally recognised with all plant diseases that unhealthy surroundings have much to do with the invasion by bacteria and other fungi. But perhaps the most important factor will prove to be the temperature. The slow growth at the higher temperatures (and the appearances of the growths in test tube cultures indicated a clearer slime at comparatively high temperatures) will, I think, explain the better qualities of the gums produced in arid regions. But on the other hand it is possible, and analogy supports this contention, that there is a class of bacteria which produce the soluble gums, each characteristic gum being formed by a different species. Future research alone will settle the question.

Since the arabin and the metarabin† gums are produced by different bacteria it is extremely probable that the pararabin gums have also their particular microbe. This investigation is

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* The partial solution may be accounted for by the treatment with acid in the autoclave during the process of eliminating the bacteria.

† Metarabin and pararabin are insoluble but swell up with water. The former is soluble in dilute alkalies, the latter in dilute acids.

in progress. Gum tragacanth will undoubtedly be shown to have a bacterial origin.

The various gums of the arabin group are not found upon Acacias only. This, however, does not militate against the bacterial origin of all these gums. Thus the gums exuded by certain Rosaceæ*, *e.g.*, cherry, peach, plum, are very similar to wattle gum, and are probably the work of bacteria such as *Bact. acaciæ* and *metarabiuum.* These fruit tree gums are being investigated.

BACTERIUM METARABINUM, n.sp.

Shape, etc.—The organism is a stout, motile, short rod, with rounded ends. The dried and imbedded bacteria when taken from cultures on saccharose-potato agar appear as oval cells, measuring 0·8 : 1·2 μ, and as rods measuring 1 : 2 μ. On the oval cells the flagella are generally terminal, but as the cell grows longer many peritrichous flagella can be seen, up to seven have been counted; but as the growths on agar are cohesive many more may occur on the cells. Staining by Gram's method is negative, and spores are not formed.

Temperature, etc.—The optimum temperature is about 30°. At 37° the growth is less than at 22° or 15°. The bacterium is aërobic, but grows slightly under anaërobic conditions.

Nutrient agar plate.—The colonies are dirty-white, raised and rough. When magnified they appear rounded, irregular or lobular, with a smooth edge and convoluted, puckered or crinkled centre. The deep colonies appear moruloid or very irregular, as if several colonies had fused together at different parts. As the colony increases in size the colour deepens to a yellowish-buff.

Glucose-gelatine plate.—The colonies are ivory-white, dry and puckered, and have a very cohesive consistency; it is almost

* The slime-flux or mucilage-flux and the gum-flux of certain trees have been ascribed to the action of certain Ascomycetes by Ludwig and by Beijerinck. In view of my research it is possible that in some cases at least these ascomycetes have appeared in the flux at a period subsequent to its formation by bacteria.

impossible to pick up a fragment of the young colony with the needle, the whole colony comes away. Microscopically, the colonies are dark, very coarsely granular and crinkled. The margin is indefinite on account of the consumption of the medium. The deep colonies are granular and very irregular. The colour deepens to a yellowish tint and the medium is slowly liquefied.

Nutrient gelatine plate.—As on glucose-gelatine, but the growth is poorer.

Nutrient agar stroke.—A narrow, slightly raised, rough, dry, glistening, lobular, yellowish-white growth forms at 30°; it adheres firmly to the medium. The condensed water carries a film from which the rough growth spreads upwards.

Glucose-gelatine stroke.—A translucent white, very wrinkled, rough, dry, dull growth, with an irregular margin is formed at 22°. A pit forms at the base of the stroke owing to the consumption of the gelatine at the place of strongest growth. The medium slowly liquefies and gravitates, producing an appearance like the clear condensed water of an agar tube. The culture coheres as a wrinkled skin. On gelatine the colour is always white or very pale yellow; while on agar it changes from white to yellow or buff.

Glucose-gelatine stab.—The stab becomes filiform-tuberculate. The nail-head is sunken and a rough growth lines the surface of the depression. Below the consumed medium the gelatine may be slightly and locally liquefied. Gas bubbles are formed in the medium; the gas formation is more pronounced in saccharose-gelatine, on plates of saccharose-potato agar, and in saccharose-potato extract.

Nutrient gelatine stab.—As in glucose-gelatine but poorer.

Saccharose-potato agar stroke.—The appearance of the culture depends upon the incubation temperature. At 30° in 24 hours the growth is luxuriant and has spread over the greater portion of the slope. It is undulating, much raised, glistening, granular and apparently very slimy. The colour is the same as the medium, viz., buff or very light oak. The condensed water has been absorbed. In 48 hours the growth has spread entirely over

the agar surface. At 37° in 24 hours the growth is broad, raised, glistening, with a translucent margin and opaque centre. The culture is apparently gummy. The condensed water has a film. In 48 hours the growth by reflected light appears homogeneous and the colour of the medium. At 22° in 24 hours the growth is broad, whitish, very rough and undulating. The margin is more glistening than the centre. The condensed water carries a film. In 48 hours the growth is mottled and pale buff. At 15° the growth is as at 22°, but less luxuriant. On this medium at 22° the growth is very like that of *Bact. acaciæ*, but differs in having a lumpy (gelatinous) consistency, while that of *Bact. acaciæ* is homogeneous. At the higher temperature the differences are more pronounced.

Potato.—A dry, pale buff scattered growth spreads irregularly over the medium. It is raised and becomes undulating. A glistening appearance begins at the margin and spreads inwards as the colour deepens to orange-yellow.

Bouillon.—The medium becomes turbid and forms a strong surface ring and a floating puckered film. The indol reaction was obtained, but nitrite is not produced as with *Bact. acaciæ*. In nitrate-bouillon the nitrate is reduced to nitrite.

Milk.—The medium slowly coagulates and the reaction is faintly acid.